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Elimination of Lyme Disease Spirochetes from Ticks Feeding on Domestic Ruminants[▽]

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To determine whether and which spirochetes are cleared from *Ixodes ricinus* ticks during feeding on ruminants, ticks were removed from goats and cattle grazing on tick-infested pastures. Although about a quarter of ticks questing on the pasture were infected by spirochetes, no molted ticks that had previously engorged to repletion on ruminants harbored Lyme disease spirochetes. *Borrelia miyamotoi* spirochetes, however, appear not to be eliminated. Thus, the more subadult ticks are diverted from reservoir-competent hosts to zooprophylactic ruminants, the smaller the risk of infection by Lyme disease spirochetes is.

Various vertebrates serve as reservoir hosts for the tick-borne agents of Lyme disease. A competent reservoir host acquires Lyme disease spirochetes when an infected tick feeds on it and maintains them to become and remain infectious for feeding ticks (10). It appears that each of the seven genospecies of *Borrelia burgdorferi* sensu lato prevalent in Central Europe is associated with particular reservoir hosts. Whereas rodents serve as a reservoir for *B. afzelii* and the recently differentiated but not yet validated "*Borrelia bavariensis*," birds maintain *B. garinii* and *B. valaisiana* (3, 4). *B. lusitaniae* and *B. spielmanii*, on the other hand, seem to be limited to lizards and dormice, respectively (9, 12, 13). Ticks harboring rodent-associated spirochetes from their larval blood meal may lose the infectious burden when feeding as nymphs on a bird and vice versa (5). It appears that solely *B. burgdorferi* sensu stricto constitutes an intermediate position, since it may be perpetuated by birds and rodents (10, 11). As a generalist, *B. burgdorferi* sensu stricto appears to be less efficiently adapted to rodents than is the specialist *B. afzelii*. A host that is competent for one genospecies seems less competent or incompetent for another.

The Central European vector tick, *Ixodes ricinus*, not only feeds on small animals. Wild ruminants, such as red, roe, and fallow deer, are frequently infested by all three stages of this tick (2, 6, 15). Interestingly, virtually no spirochetes were detected microscopically in ticks recovered from shot deer. On pastures, where domesticated ruminants graze at an extensive density, spirochetal infection in questing ticks is less prevalent than in nearby nonpastured sites (8). These ruminants appear to exert a zooprophylactic effect. Ruminants, although feeding numerous ticks, appear to be incompetent hosts for Lyme disease spirochetes. It is not known whether the incompetence of ruminants eliminates spirochetes in the feeding tick and whether it extends to each of the Lyme disease genospecies.

To determine whether and which spirochetes are cleared from ticks feeding on ruminants, ticks were removed from goats and cattle grazing on tick-infested pastures and examined at various developmental stages for Lyme disease genospecies and *B. miyamotoi.* Infection rates in ruminant-derived ticks were compared to that in ticks questing on the pastures.

The cattle study site was located southwest of the city of Flensburg, Germany, at the German-Danish border. The former training area of the German armed forces is used as low-intensity pasture, covering about 400 ha. Galloway cattle, in herds of mother cows, and Konik horses are allowed to graze year-round and are rotated on grazing patches. Most cattle which were examined for feeding ticks grazed in a 40-ha area which has been pastured since October 2004 and from which cattle and horses are excluded each year from April through June to permit rare plants to bloom and seed. The approximate grazing density of 0.25 livestock units (LU)/ha throughout the rest of the year fails to keep the vegetation short. The goat site was located about 50 km southeast of Stuttgart, Germany, near the village of Gruibingen in the Swabian highlands. Beech and juniper heath characterize the southern-facing mountain slopes, where goats were allowed to graze in a rotating regime during the vegetation period. The sites were in use as pastures for different lengths of time, with the oldest dating back to 2004.

To obtain feeding ticks from cattle and goats, two approaches were used. For the yearly blood sampling in the spring, cattle were corralled into squeeze chutes. The head of each animal was examined for ticks. Feeding ticks were carefully removed with forceps, and replete ticks were gently rubbed off onto a sheet of fabric positioned under the cow's head. From April through October 2006 and 2007 and in May of 2008, tame goats were examined individually for feeding ticks monthly and feeding ticks were removed with forceps. Ticks recovered from an individual animal were confined in screened vials and stored at 22°C to permit molting and/or until they were examined for spirochetes. Questing ticks were collected monthly from April through October 2008 in the cattle site and from April through October 2005 through 2007 at the goat site. They were collected by means of a flannel flag, identified to stage and species by microscopy, and preserved in 80% ethanol. To detect and identify the various spirochetes that may be present in questing or host-derived ticks, DNA from individual ticks was isolated, and a 600-nucleotide fragment of the gene carrying the 16S rRNA gene was amplified by

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TABLE 1. *Borrelia* genospecies detected in *I. ricinus* ticks that had engorged as larvae, nymphs, or adults on goats or cattle

Host	No. of host animals	Ticks					% of infected ticks harboring *Borrelia* sp.[b]				
		Stage	State	No. examined	% infected	% infected by LD[a] spirochete	*afz*	*gar*	*val*	*lus*	*miy*
Goats	10	Larva	Engorged	17	0.0	0.0					
	2	Nymph	Molted	2	0.0	0.0					
	80	Nymph	Engorged	379	1.6	0.8	16.7	0.0	16.7	16.7	50.0
	22	Adult	Molted	37	0.0	0.0					
	68	Adult	Engorged	306	2.9	1.6	33.3	11.1	0.0	11.1	44.4
Cattle	36	Nymph	Engorged	96	1.0	0.0	0.0	0.0	0.0	0.0	100
	42	Adult	Molted	319	0.9	0.0	0.0	0.0	0.0	0.0	100
	10	Adult	Engorged	30	0.0	0.0					
Total	144[c]			1,186	1.4	0.7	23.5	5.9	5.9	11.8	52.9

[a] LD, Lyme disease.
[b] *afz*, *B. afzelii*; *gar*, *B. garinii*; *val*, *B. valaisiana*; *lus*, *B. lusitaniae*; *miy*, *B. miyamotoi.*
[c] Value is not the sum of the above numbers because individual hosts were infested by various tick stages.

nested PCR and sequenced as described previously (12). This method detects as little as a single spirochete even in the presence of tick and ruminant DNA. Each resulting sequence was compared with sequences of the same gene fragment representing various spirochetal genospecies. The following sequences were used for comparison: GenBank accession numbers X85196 and X85203 for *B. burgdorferi* sensu stricto, X85190, X85192, and X85194 for *B. afzelii*, X85193, X85199, and M64311 for *B. garinii*, X98228 and X98229 for *B. lusitaniae*, X98232 and X98233 for *B. valaisiana*, AY147008 for *B. spielmanii*, and AY253149 for *B. miyamotoi*. A complete match, permitting no more than two nucleotide changes, was required.

Ticks removed while feeding on cattle or goats were examined for spirochetal DNA by nested PCR. Nineteen larvae were obtained during their feeding on goats, but none of the 17 engorged larvae and 2 resulting nymphs contained spirochetal DNA (Table 1). Of the 416 nymphal ticks that were obtained from 80 goats, only 9% developed to the adult stage, because most of the nymphs were only partially fed. None of the 37 resulting adults contained spirochetal DNA. However, three partially fed nymphal ticks were infected by Lyme disease spirochetes (0.8%), one each by *B. afzelii*, *B. valaisiana*, and *B. lusitaniae*. In three additional nymphs (0.8%), DNA of *B. miyamotoi* was detected. Of the 415 engorged nymphal ticks obtained from 42 cattle, as many as 319 (77%) molted to the adult stage, because mostly replete ticks had been collected from the cattle's heads. None of the cattle-derived molted ticks harbored DNA of Lyme disease spirochetes. Four ticks, a nymph and three adults (one male and two females), contained DNA of *B. miyamotoi*. Of 306 partially engorged females removed from 68 goats, spirochetal DNA was detected in 9 females (2.9%); three harbored *B. afzelii*, four *B. miyamotoi*, and one each *B. garinii* and *B. lusitaniae*. In addition, 30 females which had fully engorged on cattle were tested for spirochetal DNA after egg-laying. None of these contained spirochetal DNA. Although DNA of Lyme disease spirochetes was detected in a rare partially fed tick, no molted tick that had previously engorged to repletion on a ruminant was infected by Lyme disease spirochetes. In contrast, *B. miyamotoi* appears to be present in ruminant-fed ticks regardless of their feeding state.

The prevalence of spirochetal infection was determined in questing ticks collected on the pastures on which the cattle or the goats had roamed. A third of the nymphs and nearly a fifth of the adult ticks that quested on the cattle pasture in northern Germany contained spirochetal DNA (Table 2). The majority of these nymphs and half the infected adults were infected by *B. afzelii*. About a fifth of the nymphs and a quarter of the adult ticks questing on the goat pastures in southern Germany were infected by spirochetes. *B. afzelii* and *B. lusitaniae* infected most of these ticks. Thus, the cattle and goats in the study sites must have been exposed to numerous vector ticks infected by spirochetes.

Ticks infected by Lyme disease spirochetes appear to lose their infection when feeding on goats or cattle. If the blood

TABLE 2. Relative prevalences of *Borrelia* genospecies in questing nymphal and adult *I. ricinus* ticks sampled on goat or cattle pastures in Germany

Grazing animals on pasture	Ticks			% of infected ticks harboring *Borrelia* sp.[a]								
	Stage	No. examined	% infected	*afz*	*gar*	*val*	*bur*	*lus*	*spi*	*bis*	*miy*	>1 genospecies
Goats	Nymph	557	17.2	41.7	6.3	5.2	4.2	36.5	0.0	0.0	8.3	2.1
	Adult	511	25.0	13.3	7.0	6.3	3.9	61.7	0.8	0.0	10.2	3.1
Cattle	Nymph	413	32.4	90.3	0.7	0.7	0.0	0.0	0.0	4.5	6.0	2.2
	Adult	67	17.9	50.0	16.7	8.3	0.0	0.0	0.0	16.7	8.3	0.0

[a] *afz*, *B. afzelii*; *gar*, *B. garinii*; *val*, *B. valaisiana*; *bur*, *B. burgdorferi*; *lus*, *B. lusitaniae*; *spi*, *B. spielmanii*; *bis*, *B. bissettii*-like (1, 7); *miy*, *B. miyamotoi.*

meal on ruminants had no effect on the spirochetal burden, about 130 and 70 of the analyzed nymphs derived from cattle and goats, respectively, should have contained spirochetal DNA. The two infected cattle-derived ticks harbored solely spirochetes not related to those causing Lyme disease. Most of the ticks removed from goats were partially fed and appeared to be somewhat more likely to contain spirochetal DNA. Whether the detected DNA indicates viable spirochetes is not known. Either feeding on goats fails to eliminate spirochetes as effectively as does a blood meal on cattle or, more plausibly, engorgement to repletion is required for a complete elimination of DNA from Lyme disease spirochetes. If no spirochetal DNA is detected, the tick cannot contain viable spirochetes and thus is not infectious in its host-seeking stage.

Wild and domestic ruminants appear to be reservoir incompetent for Lyme disease spirochetes. They do not constitute reservoirs for this pathogen, because no larval tick feeding on them acquires Lyme disease spirochetes. Of 176 engorged *I. ricinus* larvae or resulting nymphs that had been collected from roe, fallow, red deer, and wild sheep in a Central European site in an earlier study, spirochetes were detected by dark-field microscopy in only two ticks (6). Similarly, only 2 of nearly 200 *Ixodes dammini* nymphs resulting from larvae that had engorged on white-tailed deer in northeast America contained spirochetes detectable by direct immunofluorescence (15). No spirochetes were detected by phase-contrast microscopy in more than 200 Swedish nymphs that derived from roe-deer-fed larvae (2). Considering that *B. miyamotoi* morphologically resembles Lyme disease spirochetes, it is likely that all of the spirochetes detected microscopically in ruminant-derived ticks during these earlier studies were not related to *B. burgdorferi* sensu lato. Not only do larvae fail to acquire Lyme disease spirochetes from ruminants, but infected nymphs also appear to lose their spirochetal load when feeding on these animals, as the present study demonstrates. In ticks that had fully engorged on cattle, the only spirochetal DNA that was detected was that of *B. miyamotoi*. Also, the American strain of *B. miyamotoi* was discovered in larvae resulting from field-collected adult females that had routinely been fed on sheep (14). The previous observation that the prevalence of *B. miyamotoi* in a cattle pasture was not significantly reduced compared to that in the nonpastured site nearby further exemplifies the differential effect of ruminants on these two kinds of spirochetes (8). Whereas Lyme disease spirochetes are eliminated when their tick vector feeds on a ruminant, *B. miyamotoi* appears not to be affected by such a blood meal.

Ruminants reduce the prevalence of infected ticks on a pasture. For the present study, sites were chosen that had only recently come into use as pastures and where cattle were excluded during the peak season of tick activity. The spirochetal prevalence was thus similar to that in the surrounding areas where no domestic ruminants roamed (data not shown) and permitted us to compare infection rates before and after the blood meal on ruminants. The effect of the grazing schedule and of the grazing duration that is required to result in reduced prevalence still needs to be determined. Domestic ruminants employed in landscape management appear to exert their zooprophylactic effect in multiple ways, by eliminating spirochetes from vector ticks feeding on them and by reducing the ecotonal vegetation, thereby limiting coverage and food sources of reservoir hosts while simultaneously rendering the microclimate less suitable for vector ticks. This study's observations indicate that Lyme disease spirochetes are eliminated from the tick during its blood meal on a ruminant. The mechanism by which Lyme disease spirochetes are cleared during the tick's blood meal is under investigation. Evidently, Lyme disease spirochetes are destroyed in a way that renders their DNA no longer detectable by means of nested PCR. A simulation model indicates that the availability of incompetent hosts for subadult tick stages would reduce the prevalence of infection (16). Therefore, the more subadult ticks are diverted from reservoir competent birds or mice to incompetent ruminants, the smaller the risk of infection with the agent of Lyme disease is.

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